Efficient Expansion of Suspension CHO Cells in Corning® 5L Erlenmeyer Flasks

Application Note

CORNING

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Introduction

Erlenmeyer flasks are commonly used for expansion of a variety of suspension cell lines for bioprocessing applications. Corning provides disposable Erlenmeyer flasks ranging from 125 mL to 3L in size. Corning developed a 5L Erlenmeyer flask with the same footprint as a traditional 3L Erlenmeyer flask.

The 5L shape has been optimized for increased gas exchange compared to the more traditional Erlenmeyer flask designs. Due to gas exchange limitations, most traditionally shaped Erlenmeyer flasks can only accommodate approximately one third of the stated volume of the vessel during culture. Corning's 5L shape design allows for the culture volume to be increased to one half of the stated volume of the flask, resulting in a greater number of cells cultured in the same footprint as a 3L flask.

In this study, we describe a protocol to scale up suspension CHO cells from 40 mL to 15L of total volume in just 12 days using Corning 5L Erlenmeyer flasks. Furthermore, we demonstrate that Corning 5L Erlenmeyer flasks provide a more favorable culture environment (higher oxygenation and lower lactate metabolite accumulation) and support a higher yield of viable CHO cells when relative to comparable 5L Erlenmeyer flasks.

Materials and Methods

Culturing conditions

CHO 5/9 m alpha3-18 cells (ATCC CRL-10154) were adapted to suspension culture in EX-CELL® CD CHO Fusion medium (Sigma 14365C) using the protocol recommended by the medium manufacturer. Adapted cells were cryopreserved at 1 x 10^7 cells/vial and subsequently used for these studies. One vial of cells was thawed directly into 40 mL of EX-CELL® CD CHO Fusion medium in a 125 mL Corning Erlenmeyer flask (Corning 431143) and rotated at 120 rpm. Cells were considered fully recovered from cryopreservation when they consistently reached >2 x 10^6 cells/mL on day 3 with cell viability >95% (after 3 to 4 passages). The VWR mini-shaker (VWR 97109-890) was used for 125 mL flasks (120 rpm agitation rate) and the Multitron Pro incubation shaker (Infors HT) with a 25 mm orbit was used for 1L and 5L flasks (90 rpm agitation rate) for all experiments.

Cell Expansion

The cells were sequentially passaged every 3 to 4 days at a seeding density of 3 x 10^5 cells/mL from a Corning 125 mL Erlenmeyer flask (Corning 431143, 40 mL working volume) to a Corning 1L Erlenmeyer flask (Corning 431147, 300 mL working

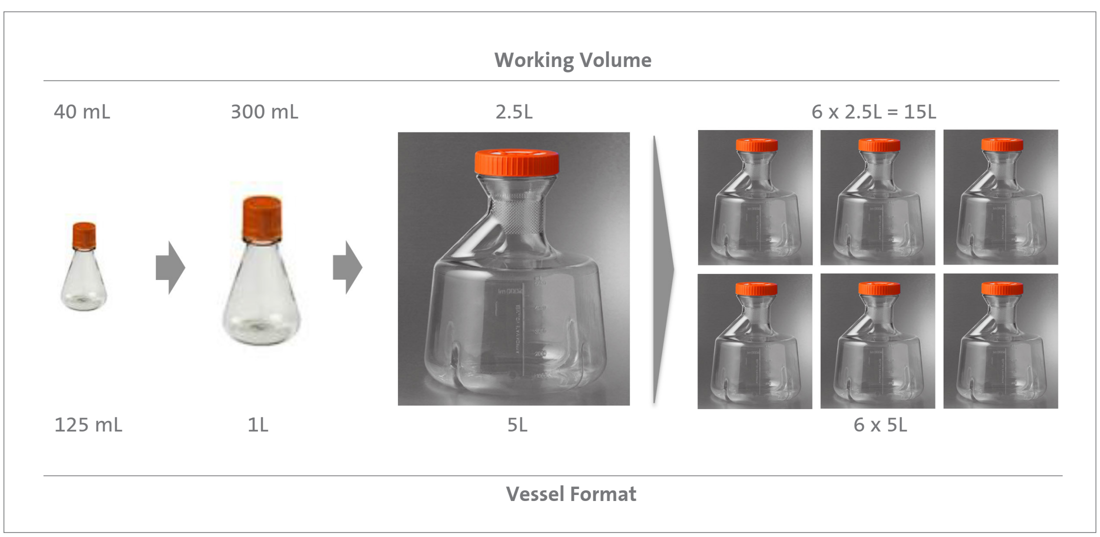

Figure 1. Cell scale-up protocol for Corning Erlenmeyer flasks. The protocol provides an efficient scale up solution for suspension CHO cells from 40 mL to 15L of total volume in 12 days using Corning Erlenmeyer flasks.

volume), and finally to a Corning® 5L Erlenmeyer flask (Corning 431685, 2.5L working volume), as illustrated in Figure 1. Cells were enumerated using the Vi-CELL™ Cell Viability Analyzer (Beckman Coulter), and assessed for viability using the trypan blue exclusion assay. This study was repeated 3 independent times with one or two replicates per study/condition.

Benchmarking

Cells cultured in 5L Erlenmeyer flasks were seeded at 3 x 10^5 cells/mL into 3 different flasks: a Corning plain bottom 5L Erlenmeyer flask (Corning 431685), a Corning baffled bottom 5L Erlenmeyer flask (Corning 431684), and a comparable baffled bottom 5L Erlenmeyer flask. Cells were enumerated daily as described above. Spent media analyses were performed using the Nova BioProfile® BP400 analyzer. The experiment was repeated 3 independent times with one or two replicates per study/condition. ANOVA was used for the analysis with p-values of <0.05 considered statistically significant. Tukey's test was used to differentiate between the conditions.

Results and Discussion

Figure 1 illustrates the scale-up protocol using Corning Erlenmeyer flasks. This protocol allowed for efficient expansion of CHO cells from 40 mL to 15L of total volume in just 12 days, providing an easy, scalable method for generating a large number of cells. As shown in Figure 2, a comparable growth rate was observed between Corning 1L and 5L Erlenmeyer flasks despite the difference in vessel/total volume ratio (3.3 for 1L flasks and 2 for 5L flasks) and vessel shape. The scalability between the different sized vessels is important to ensure the translation of optimized protocols, such as feeding strategy, from small to larger scale flasks.

Next, we compared CHO cell expansion in the Corning versus comparable 5L Erlenmeyer flasks. As shown in Figure 3, the cell yield was comparable for all flasks up to day 5. In contrast, a statistically significant increase in cell yield was observed for both Corning flasks relative to a comparable flask after day 6. The increase was more pronounced with baffled versus plain bottom Corning flasks. Cell viability was comparable for all three growth conditions (Figure 4).

Spent media analyses were performed to elucidate the difference in cell yield observed with Corning versus comparable flasks. As shown in Figure 5A, the dissolved oxygen levels in the spent medium were higher for Corning relative to the comparable flasks on days 2 to 8. In contrast to cells cultured in the comparable flasks, cells cultured in the Corning flasks during days 7 to 9 were found to utilize lactate, leading to less accumulation of this toxic metabolite (Figure 5C). Taken together, these data suggest that Corning 5L Erlenmeyer flasks provide a more favorable environment (higher dissolved oxygen, less lactate accumulation) for CHO cell growth relative to the comparable flasks, leading to higher cell yields. Comparable metabolic profiles were observed for glucose, glutamine, and ammonium for cells cultured in all tested flasks (Figures 5B, D, and E, respectively).

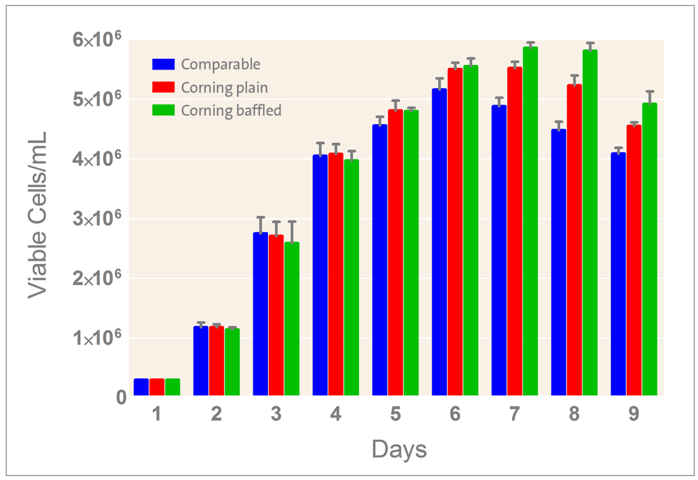

Figure 3. Improved CHO cell yield with Corning 5L Erlenmeyer flasks. A statistically significant difference in viable CHO cell yield was observed between Corning and comparable 5L Erlenmeyer flasks on day 6 (p=0.014) and between all three flask conditions on days 7 to 9 (p=0.0001) using ANOVA and Tukey's test analyses.

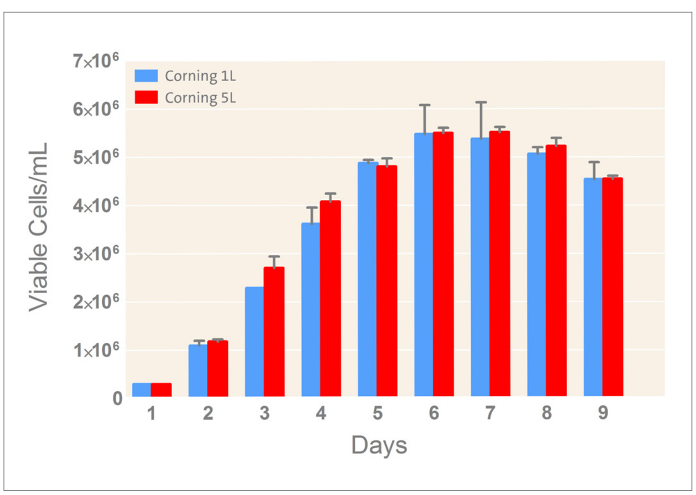

Figure 2. Scalability of Corning Erlenmeyer flasks. Comparable viable CHO cell yield/mL was observed in Corning 1L and 5L Erlenmeyer flasks throughout the 9-day culture period.

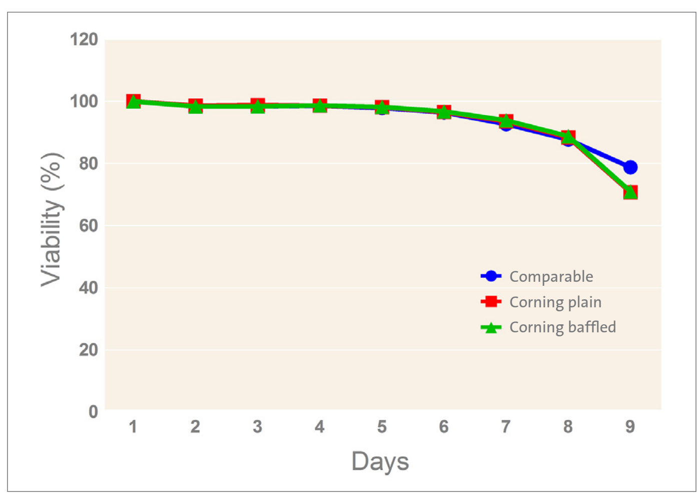

Figure 4. Comparable CHO cell viability was observed in Corning and comparable 5L Erlenmeyer flasks.

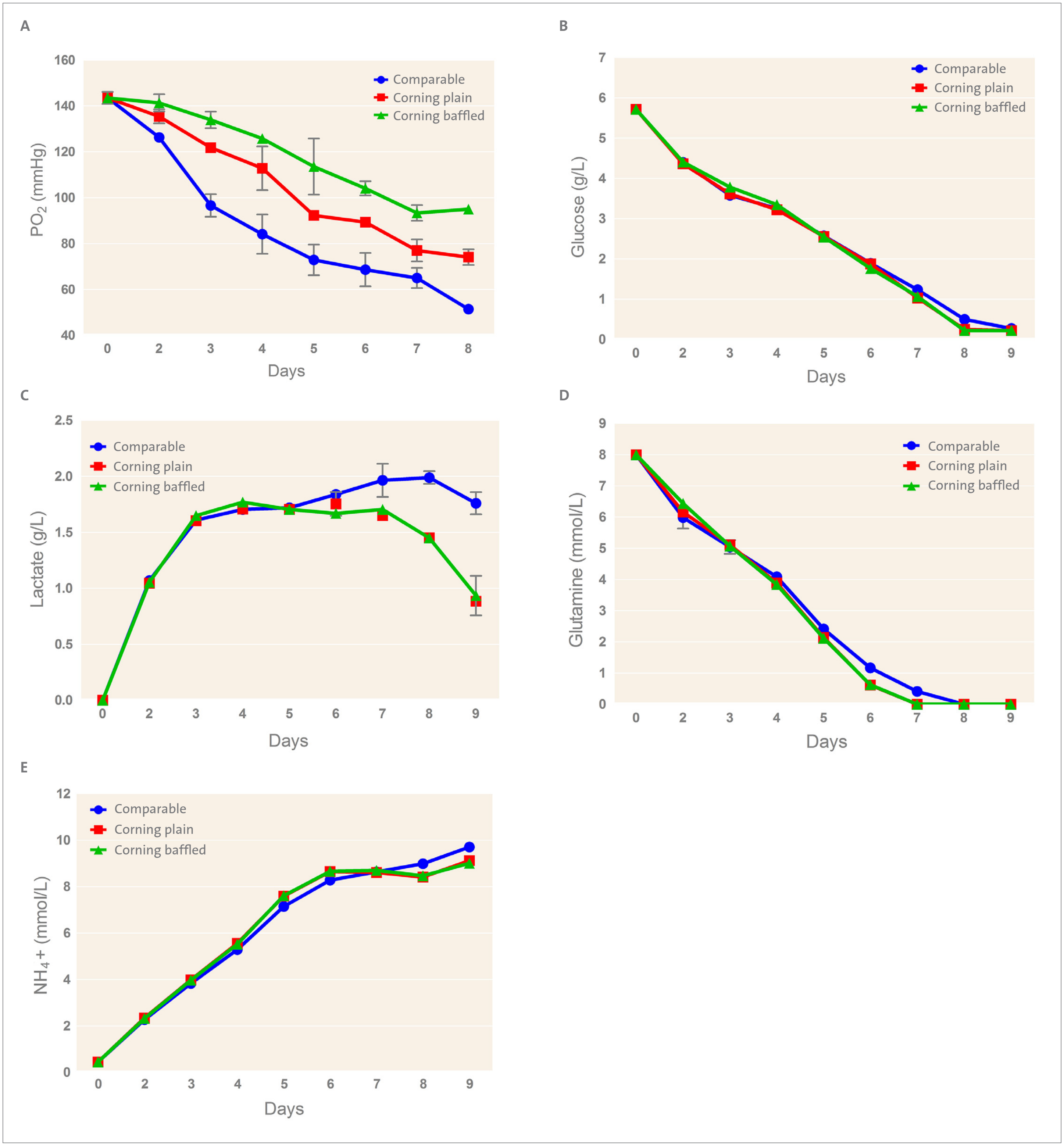

Figure 5. Key nutrient/metabolite profiles in spent media for Corning® and comparable 5L Erlenmeyer flasks. Spent media analyses showed statistically higher PO_2 (A) on days 2 to 8 and statistically lower lactate levels (C) on days 7 to 9 due to lactate consumption (metabolic shift) in Corning relative to comparable 5L flasks. Glucose consumption (B), glutamine consumption (D), and ammonium accumulation (E) were comparable between all flasks. For PO_2 (panel A) the p value ranged from 0.001 to 0.028 depending on the day of culture; for lactate (panel C) the p value was 0.068, 0.001, and 0.008 on days 7, 8, and 9, respectively.

Conclusions

- Corning® 5L Erlenmeyer flasks can be used to scale up suspension CHO cells from 40 mL to 15L of total volume rapidly and efficiently.
- A statistically higher CHO cell yield was observed with Corning 5L Erlenmeyer flasks relative to a comparable flask.
- Spent media analyses demonstrated statistically higher levels of dissolved oxygen and lower levels of lactate in Corning 5L Erlenmeyer flasks relative to a comparable flask, suggesting that the Corning flasks provide a more optimal culture environment for CHO cells under these conditions.

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